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Calculation of LDL-Cholesterol vs. Direct Homogenous Assay

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Background: Low-density lipoprotein cholesterol (LDLc) can be calculated or measured directly and their accordance is the subject of controversy. *Objectives:* The aim of this study was to identify the independent predictors of LDLc, to formulate the best equation for calculating LDLc and to evaluate the validity of it and the published formulas, including the new method with adjustable coefficient. *Methods:* The profile of serum lipids and (apo)lipoproteins of 310 subjects was used to determine the most accurate formula for calculating serum LDLc. Serum lipids, lipoproteins and apolipoproteins were measured by enzymatic, new homogenous and immunoturbidometric methods, respectively. *Results:* Multiple linear regression analysis indicates that total cholesterol, apoB, HDLc and triglyceride are independent predictors of LDLc. We proposed four new formulas to calculate LDLc. As total cholesterol (TC) is the major determinant of LDLc, it can be estimated simply as 0.545 of total cholesterol. Inclusion of HDLc, triglyceride, apoB and a constant value improved the equation slightly. The equation of: LDLc (mg/dl) = 0.75 TC − 0.5 HDLc − 0.1 TG had the lowest mean and SD of difference among all the methods examined here. LDLc was also calculated by the new modified Friedewald's equation using adjustable factor from Martin's table, but it did not improve the results significantly. LDLc gap was correlated significantly and positively with triglyceride and negatively with cholesterol or its subfractions. *Conclusions:* Our data suggest the simplest formula: LDLc = 0.545 TC or a more detailed: LDLc = 0.75 TC − 0.5 HDLc − 0.1 TG be used for calculating serum LDLc. J. Clin. Lab. Anal. 31:e22057, 2017.

Key words: ApoB; cholesterol; HDLc; homogenous assay; LDLc

INTRODUCTION

Coronary artery disease (CAD) is a multifactorial disease with over 250 known risk factors (1). Nevertheless, the independent and causative correlation has been confirmed only for seven major risk factors (2). Serum total and/or low-density lipoprotein cholesterol (LDLc) is the strongest marker for atherosclerosis (3). According to the National Cholesterol Education Program Adult Treatment panel (NCEP-ATP), the level of serum LDLc has been identified as the primary basis for risk assessment, classification and treatment of patients with hyperlipidemia (4). Beta-quantification using ultracentrifugation is the reference method to measure LDLc, but it is an expensive and time consuming technique (4). Most clinical laboratories routinely calculated LDLc using Friedewald equation (5) as: LDLc = total cholesterol- HDLc- TG/5. The equation assumes that the ratio of cholesterol to triglyceride in VLDL fraction is one-fifth. Although the calculation correlates well with the measured LDLc, it has some limitations. This formula assumes: the ratio of total triglyceride to VLDL cholesterol (VLDLc) is constant in all samples; it is not valid for samples with triglyceride more than 400 mg/dl or in patients with dysbetalipoprotenemia. It has also limited to use in type-II diabetes mellitus, nephrotic syndrome and alcoholic patients (6–8). In samples with low triglyceride and high total cholesterol, calculation also may overestimate LDLc concentration (9).

In the last decade, several homogenous assay methods have introduced to measure LDLc directly (4). Although it is better to measure LDLc directly,

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calculation is free of charge and most laboratories continue to use that method. There are several different formulae to calculate serum LDLc (8–20). The number of these formulae has been increased because the homogenous direct assay of LDLc is easier and more available than beta-quantification. In the more recent method of Martin et al., the coefficient of triglyceride in Friedewald equation is assumed to be adjustable relative to nonHDLc and triglyceride (21,22). The validity of the original Friedewald and the new methods have been questioned in recent studies (21–26). Advocacy for adaption of a new method requires independent verification in a multiple data set. So, in this study, linear regression analysis was performed to identify the independent predictors of LDLc and formulate the best equation for calculating LDLc. In addition, the data of our patients were used to evaluate the validity of the new equations and other formulas cited in the literature.

EXPERIMENTAL DESIGN, SUBJECTS AND BIOCHEMICAL MEASUREMENTS

The subjects were 148 men and 152 women aged 35–76 years who were referred to Zahra hospital of university of Mazandaran. All measurements were done on fresh serum except that of apolipoprotein B100, homogenous LDLc and HDLc, which was stored at −70°C before analysis for maximum of 6 months. Serum total cholesterol and triglycerides (TG) were measured enzymatically by the CHOD-PAP and GPO-PAP methods, respectively (Pars-Azmon Inc., Tehran, Iran). LDL cholesterol (LDLc) and high density lipoprotein cholesterol (HDLc) were determined using the new homogenous assay (Pars-Azmon Inc.). Unesterified total cholesterol and unesterified HDLc were measured by the same kits but without the enzyme cholesterol esterase. Esterified total cholesterol and esterified HDLc were calculated by subtractions of unesterified fractions from total cholesterol and HDLc (4,23). ApoB100 and apoAI were assayed by immunoturbidometric methods (Diagnosis Inc., Germany). Inter- and intra-assay coefficients of variance were <5% for all measurements. All other biochemical and hematological parameters were measured by routine laboratory methods.

Statistical Analysis

The results are presented as the mean ± SD. All *P*-values are two- tailed and differences were considered significant if *P*-values were ≤0.05. Bivariate correlation analysis was carried out to find out the association of dLDLc with other variables. Multivariate linear regression analysis was conducted using SPSS (version 21; SPSS Inc, Chicago, IL) automatically and Excel (Microsoft Inc, Washington, DC) software manually to determine the factors of regressors. The student's *t*-test and *F*-test were used to compare the mean of difference and the mean of standard deviations of the methods, respectively.

RESULTS

Major Determinants of LDLc

In the preliminary step, bivariate correlation analysis was performed to establish the major determinants of serum LDLc. Table 1 shows the characteristics of the study population and also indicates that LDLc has a significant association with total cholesterol, HDLc, triglyceride and aopB100. The correlations did not change when total cholesterol has been fractioned into esterified and unesterified. Unless, unesterified HDLc showed more and esterified fraction had less association with LDLc.

Multivariate Linear Regression Analysis

Multiple linear regression analysis with a stepwise approach was performed to predict LDLc using SPSS software. Direct LDLc was entered as dependent and all other biochemicals as independent variables. The results were presented as the unstandardized and standardized coefficients β, partial and multiple correlation coefficients in Table 2. The unstandardized coefficients β indicate the actual coefficients of variables in the regression equation. The standardized coefficient β is associated with 1 SD change in the independent variable, indicates the importance of each regressor and

TABLE 1. The Characteristics of the Study Population and the Correlation Coefficients of LDLc with Other Biochemicals

		LDLc correlations	
Variables	Mean ± SD	(*r*)	*P*
Age	57.4 ± 10.1	−0.042	0.481
Sex (M:F)	152:148	0.013	0.857
Total cholesterol	186.3 ± 46.9	0.898	0.001
Unesterified	72.9 ± 26.7	0.647	0.001
Esterified	114.3 ± 39.2	0.705	0.001
ApoB100	118.6 ± 39.5	0.718	0.001
ApoAI	173.5 ± 50.1	0.139	0.040
HDLc	41.5 ± 10.8	0.405	0.001
Unesterified	12.0 ± 4.2	0.610	0.001
Esterified	29.5 ± 9.4	0.200	0.001
Triglyceride	193.1 ± 132.3	0.141	0.015
Glucose	117.9 ± 49.8	−0.021	0.715

Bivariate correlation analysis was performed using SPSS software.

TABLE 2. Multivariate Linear Regression Analysis

Included variables	Mean ± SD	Unstandardized coefficient (β)	Standardized coefficient (β)	*r*	*R*	*P*
+ Total cholesterol	186.3 ± 46.9	0.639 ± 0.026	1.150	0.986	0.990	0.001
+ Triglyceride	193.1 ± 132.3	−0.102 ± 0.011	−0.175	−0.543	0.993	0.001
+ HDLc	41.5 ± 10.8	−0.440 ± 0.073	−0.174	−0.403	0.994	0.001
+ ApoB100	118.6 ± 39.5	0.153 ± 0.029	0.126	0.354	0.995	0.001

In any model, a new variable was added to the previous variable(s) and the results of the last model with four parameters has presented without a constant value. *R*: Multiple correlation coefficient and *r*: partial correlation coefficient.

was highest for total cholesterol. The results showed that only serum total cholesterol, HDLc, triglyceride, and aopB100 are independent predictors of LDLc. In the absence of apoB, the unstandardized coefficients β were 0.75, −0.5, and −0.1 for total cholesterol, HDLc, and triglyceride, respectively. These are also the actual coefficients of the terms involved in the Eqn-2 of Table 3. The multiple correlation coefficients (*R*) of the models were not improved by stepwise entering the four independent variables.

Deducing of the Equations to Calculate LDLc

In Table 3, we introduced four new equations and also compared the results of different formulas found in literature with direct measured LDLc. Since total cholesterol was the major determinant of LDLc, we performed the analysis with only cholesterol. The first equation has effectively a zero mean of difference. Inclusion of a constant value in the equation did not improve the formula. Automatic regression analysis with three and four regressors produced the Eqns 3 and 4, respectively:

$$\text{dLDLc} = 0.75\ \text{TC} - 0.5\ \text{HDLc} - 0.1\ \text{TG}$$
$$\text{dLDLc} = 0.65\ \text{TC} + 0.14\ \text{apoB} - 0.42\ \text{HDLc} - 0.11\ \text{TG}$$

Since the unstandardized coefficients β are far from the reality, regression analysis was performed manually with some coefficients predetermined. The coefficients of total cholesterol and HDLc can be predetermined as one manually as.

$$\text{dLDLc} = (\text{TC} - \text{HDLc}) - \text{m}(\text{TG}) + \text{b}$$

The equation was rearranged into the standard linear equation form, $y = \text{mx} + \text{b}$ as:

TABLE 3. Comparison of the Measured LDLc Relative to Calculated LDLc from Different Equations Taken from the Literature Using Data from the Present Study

	Equation	*R*	Mean of difference	Mean of SD	Ref.
1	dLDLc = 0.545 TC	0.898	−0.6	15.3	Ours
–	dLDLc = 0.615 TC − 13.9	0.898	−0.2	14.9	Ours
2	dLDLc = 0.75 TC − 0.5 HDLc − 0.1 TG	0.931	0.2	12.3*	Ours
3	dLDLc = TC − HDLc − TG/4.65	0.912	−4.9	16.9	Ours
4	dLDLc = 0.65TC + 0.14 apoB–0.42HDLc − 0.11TG	0.890	5.6	15.6	Ours
1	dLDLc = 0.75 TC − 0.6465/0.0259	0.959	−13.6	16.3	Hu et al.
2	dLDLc = TC − HDLc − TG/5	0.917	−7.4	16.6	Friedewald et al.
3	dLDLc = TC − HDLc − TG/5.2	0.919	−8.8	16.4	Delong et al.
4	dLDLc = 0.9 (TC − TG/5) − 28	0.909	−6.0	17.4	Anandarajaet al.
5	dLDLc = TC − HDLc − TG/6	0.925	−13.5	15.9	Puavilai et al.
6	dLDLc = 0.90 (TC − HDLc) − TG/8	0.927	−21.1	16.1	Tsai et al.
7	dLDLc = 0.90 (TC − HDLc) − TG/10	0.819	−47.9	26.4	Chen et al.
8	dLDLc = TC- HDLc − TG/3	0.830	17.1	23.8	Vujovic et al.
9	dLDLc = 0.996 TC − 0.985 HDLc − 0.1998 TG + 7.15	0.917	−14.4	16.5	Dansethakul et al.
10	dLDLc = 3/4 (TC − HDLc)	0.888	−7.7	16.0	de Cordova et al.
11	dLDLc (mM) = 0.41TC + 1.70apoB − 0.32 TG − 0.27	0.885	7.5	16.1	Bairaktari et al.
12	dLDLc = TC − HDLc − TG/adjustable coefficient (m)	0.924	−14.3	15.1	Martin et al.

The concentrations of all solutes are expressed as mg/dl. The mean of the correlation coefficients and mean of SD of all equations were compared to corresponding values of Friedwald equation. The mean of standard deviations of the methods were compared by *F*- test.
*The difference is statistically significant at the confidence levels of $P \leq 0.01$. The coefficient 0.0259 in the Eqn-1 was used to convert mmol/l into mg/dl.

$$dLDLc - (TC - HDLc) = -m(TG) + b$$

That is identical with:

$$dLDLc - (nonHDLc) = -m(TG) + b$$

When the value of (dLDLc – NonHDLc) is plotted against (TG), linear regression analysis gives the slope m and intercept b. The results revealed that the slope m was –0.215 if the constant value to be zero:

$$dLDLc - (TC - HDLc) = -0.215\ TG$$
$$dLDLc = TC - HDLc - 0.215\ TG$$

The equation looks very similar to the formula of Friedewald.

Comparison of Calculated LDLc Derived from Different Equations

LDLc was calculated according to twelve different published equations using data from the current study and compared with measured direct LDLc (Table 3). Correlation coefficient *R* was calculated with each equation by correlation analysis of the data. Delta LDLc was stated as measured minus calculated LDLc. The best results were chosen in terms of the highest correlation and the lowest mean and standard deviation of difference.

Equation-1 included just total cholesterol as the independent variable and have low mean of difference near to zero. Inclusion of a constant value in it did not improve the formula. The coefficients of the terms in the Eqn-3 were deduced automatically by regression analysis. This formula has the lowest mean and SD of difference among all equations. Using the factor 3 by Vojovic et al. and 10 by Chen et al. cause the highest mean and SD of difference in Eqns 7 and 8. In other equations, any adjustments had no significant effects on correlation coefficient and the mean and SD of difference. Using the adjustable factor from 180-cell table of Martin et al. also did not improve the equation significantly.

Major Determinants of LDLc Discriminate

It is assumed that the diversity of the results of calculated LDLc by different equations is a result of the coefficient of triglyceride to cholesterol in VLDL fraction. To find out the major determinant of the coefficient alpha, LDLc gap was calculated according to Friedwald equation and compared with other biochemicals. Table 4 shows that delta-LDLc is correlated significantly and positively with triglyceride and negatively with cholesterol or its subfractions. So, the ratio of triglyceride to total cholesterol or its fractions (i.e., TG/nonHDLc) has been found to be the best determinant of LDLc gap:

$$dLDLc = (TC - HDLc) - TG/\alpha$$

Rearrangement of the equation for α gives:

$$\alpha = TG/(nonHDLc - dLDLc)$$

Therefore, α is function of three variables as triglyceride, nonHDLc and dLDLc.

DISCUSSION

In the current study, the profile of serum lipids and (apo)lipoproteins was applied to identify the independent predictors of LDLc and propose the most accurate formula for calculating serum LDLc. Various equations have been derived to calculate serum LDLc by linear regression analysis (5–21). The concentration of LDLc has been measured directly by beta-quantification (5,6,21) or the new homogeneous assay (8–20). The results of Tables 1 and 2 show that serum total cholesterol, aopB100, HDLc and triglyceride are independent predictors of LDLc. As total cholesterol is the major determinant of LDLc ($\beta = 0.64$, $P = 0.001$), the Eqn-1 of Table 3 shows that LDLc can be estimated as 0.545 of total cholesterol. Inclusion of HDLc and triglyceride values improved the equation slightly. Insertion of a constant value is not logical and has not resulted any significant improvement in any equation. Table 2 shows that the unstandardized coefficient for apoB is as low as 0.15 in the regression equation. Thus, it is not recommended to measure apoB as it is not a routine procedure and did not improve the Eqns-4 and -11 significantly.

Linear regression analysis determines automatically the coefficient of total cholesterol (and also HDLc) as less than unity as is seen in Eqn-2. Although this coefficient appears unrealistic, it indicates that LDLc is correlated with total cholesterol and HDLc partly but not completely. Thus, the actual coefficients of the regressors are logical even they are less than unity. With inclusion of three regressors and without a constant value, the actual coefficients β were 0.75, −0.5 and −0.1 for total cholesterol, HDLc, and triglyceride, respectively (Eqn-2, Table 3).

The analytical methods can be compared by different criteria including correlation coefficient, the mean, and SD of difference. Coefficient of correlation is influenced by random errors, but systematic error is not affected. Therefore, the accuracy of a method should not be judged by that coefficient. The low mean and SD of difference are good criteria to access the accuracy and precision of an assay method (4). The Eqn-2 (Table 2)

TABLE 4. The Correlation Coefficient of Delta LDLc with Other Biochemicals

Variables	Coefficient of correlations	
	(*r*)	*P*
Triglyceride	0.289	0.001
Total cholesterol	−0.388	0.001
HDLc	−0.113	0.051
LDLc	−0.206	0.001
ApoB100	−0.185	0.007
Triglyceride/cholesterol	0.378	0.001
Triglyceride/HDLc	0.222	0.001
Triglyceride/nonHDLc	0.435	0.001

Bivariate correlation analysis was performed using SPSS software.

has the lowest mean and SD of difference among all the methods examined here.

The factor α in the denominator of the term triglyceride is a function of three variables; triglyceride, nonHDLc, and dLDLc. From three variables, dLDLc is a dependent variable and can be determined by two independent variables triglyceride and nonHDLc. It is supposed that the main cause of bias of LDLc calculated by equations results from using a constant factor α in the term of triglyceride (Table 4). The use of the lower and higher factor in the formula tends to under- and overestimate the true LDLc of the sample, respectively. Using the factor α as 3 by Vojovic et al. and 10 by Chen et al. caused a highest negative and positive discriminate and SD of calculated LDLc, respectively (Table 3) (15,20). Martine et al. in a study with more than 1.3 million people determined the factor α on the basis of serum triglyceride and nonHDLc (21). They introduced a 180 cell table to estimate the factor according to serum triglyceride and nonHDLc. The mean of α factor was 4.8 ± 1.1 in our study, whereas it is in the range of 3.1 up to 11.9 in the Martin's table (22). The using of this table is time consuming, but it is expected to calculate LDLc more accurately. Analyzing our data using adjustable factor from Martin's table showed that it underestimates LDLc by mean of −14.0 and SD of 15.1. Comparison the results of the new method with other formulae in Table 3 show that using an adjustable factor could not improve the equation. Other researchers also applied the adjustable factor of Martin et al. and found that the calculation will be improved only slightly (23–25). Comparison of our formulas with other formulae listed in Table 3 indicates that the Eqn-2 differs from all others significantly and provides better results.

Study Limitations

The composition of the study participants is the major limitation encountered in the current study. The majority of our patients were at high risk for cardiovascular disease who consumed statins to reduce the level of cholesterol. Automated regression analysis provides a mathematical and statistical model consistent with the data, yet it may not represent the real situation accurately.

CONCLUSIONS

It is concluded that LDLc could be calculated simply by 0.545 of total cholesterol or as: LDLc = 0.75 TC − 0.5 HDLc − 0.1 TG.

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